Reference: *Biol. Bull.*, **147**: 535–549. (December, 1974)

CLINAL AND SIZE-DEPENDENT VARIATION AT THE *LAP* LOCUS IN *MYTILUS EDULIS*

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The widespread use of enzyme electrophoresis has revealed that almost all populations are characterized by a large amount of genetic variation (Lewontin and Hubby, 1966; Selander, Hunt and Yang, 1969; Ayala, Powell and Dobzhansky, 1971; Gooch and Schopf, 1972; Clegg and Allard, 1972) and the study of many enzymes has indicated that no locus can be predicted *a priori* as being monomorphic or polymorphic. To what extent these polymorphisms are adaptive has not been resolved, and this issue is central to our understanding of how genetic variability is maintained in natural populations.

Evidence for selection may consist of (1) showing a convergence to a characteristic gene frequency after natural or artificial perturbation (Kojima and Yarbrough, 1967; Sved and Ayala, 1970; Berger, 1971), (2) demonstrating a correlation between genic and environmental variation (Koehn, 1969; Schopf and Gooch, 1971; Hamrick and Allard, 1972; Powell, 1971), or (3) finding a progressive change in gene frequencies with increasing age (Koehn, Perry and Merritt, 1971; Tinkle and Selander, 1973; Fujino and Kang, 1968). Deviations from Hardy-Weinberg proportions or persistent linkage disequilibrium may also support a selectionist interpretation (Franklin and Lewontin, 1970; Charlesworth and Charlesworth, 1973; Allard, Babbel, Clegg, and Kahler, 1972; Clegg, Allard, and Kahler, 1972). This study of genic variation at the leucine-amino-peptidase locus in *Mytilus edulis* presents evidence of types (2) and (3).

Mytilus edulis, the blue mussel, has a widespread range from North Carolina to Nova Scotia and is a prolific colonizer of a variety of habitats. Previous studies (Milkman and Beaty, 1970; Koehn and Mitton, 1972) have shown that the species is always polymorphic for three alleles at the leucine-amino-peptidase (LAP) locus and the frequency of these varies significantly from one locality to another. In addition to the three common alleles, [slow (S), medium (M), and fast (F), in order of increasing electrophoretic mobility], at least two other alleles, *R* and *G*, are found in most samples. Milkman (1971) and Milkman and Beaty (1970) have reported that *Mytilus* populations south of Cape Cod generally have a high frequency of the slow allele (around 50%) and those of Cape Cod Bay and northwards have a frequency of 15–30%. Mussels from the Nissequoque River on Long Island have a slow frequency from 11% to 14% (Koehn and Mitton, 1972). Milkman (personal communication) has correlated these variations with differences in the timing of the tidal currents. The LAP genotypes of the *Mytilus* and *Modiolus demissus* populations at the four sites studied by Koehn and Mitton were signifi-

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cantly different between sites, but were similar for the two species at each site. Thus the large scale variation found by Milkman and the smaller scale variation in the Nissequoque River support the hypothesis of selective agency in maintaining this polymorphism.

Mussels are dioecious and produce pelagic larvae which may disperse to habitats some distance from their parents. The larvae settle and after metamorphosis attach to a firm substrate and to each other by byssae, thus insuring an essentially sessile adult existence. For this reason, if a particular suite of environmental factors favors particular genotypes, we may anticipate a progressive change in gene frequency with increasing size. This study of *Mytilus* populations on the southern shore of Cape Cod Bay shows both clinal and temporal divergence in LAP allelic frequencies.

Methods and Materials

Mussels were collected from two estuaries on the northern side of Cape Cod. The first locality, Sandwich Harbor/Mill Creek, is 1 km east of the northern terminus of the Cape Cod Canal, and the second, Scorton Creek, is 2.2 km east of Sandwich Harbor. Mussels are found in channels which drain extensive salt marshes and which are inundated by tidal sea water (mean tidal range is 3 meters). *Mytilus* is found in both the subtidal and intertidal zones from the entrance of the

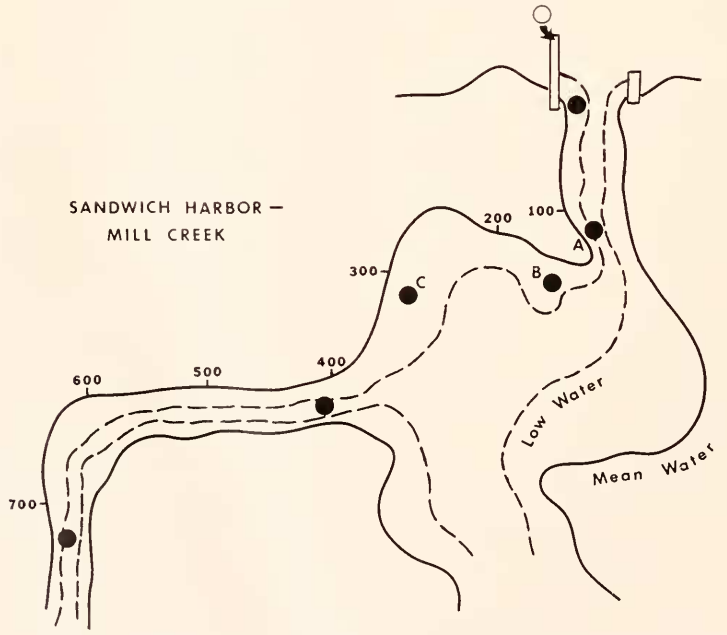

Figure 1. Sandwich Harbor. The six sites in the estuary are indicated as filled circles, and the temporary colony from the high intertidal as an open circle. The distance from the entrance measured along mean tide line is given in meters.

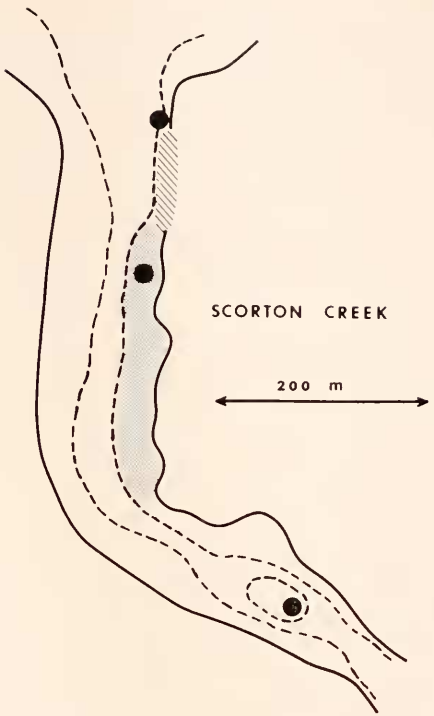

FIGURE 2. Scorton Creek. Filled circles mark the three sites. Stone boulder jetty is indicated by hatching and the extensive mussel beds by stippling.

channels into Cape Cod Bay to a point 800 meters upstream at Sandwich Harbor and 600 meters at Scorton Creek, but are not found higher than mean water. Mussels were sampled from 6 sites at Sandwich Harbor and 3 sites at Scorton Creek (see Figs 1 and 2).

Collections were also made from the intertidal zone at the jetty on the east side of northern terminus of the Cape Cod Canal, the jetty at the Sandwich Harbor entrance, and five sites around the circumference of the very large bay and estuary which comprises Barnstable Harbor (12 km east of Scorton Creek).

Mussels were maintained in the laboratory for one to three days in running sea water. Mortality was negligible prior to assay. The maximum length was measured and then a portion of the liver was homogenized in a 1 : 1 dilution of running buffer (commercial Gelman tris-barbital, pH = 8.8). This extract was spotted on Gelman cellulose polyacetate strips and electrophoresis was carried out in Gelman Trays for 45 minutes at 250 V, 2 mAmp/strip. Strips were incubated for 6 minutes in 25 ml tris-maleate buffer (0.2 M) at a pH of 5.2 (adjusted with NaOH) containing 10 mg 1-leucyl-β-naphthalamide. They were stained for 6 minutes in 25 ml tris-maleate with 25 mg of Fast Black K-salt.

TABLE I

Gene frequencies and sample sizes for all localities by size classes. Number of rare alleles, and observed and expected (Hardy-Weinberg) percentages of homozygotes (SS, MM, and FF) also given.

Size range (in mm)	Number in sample	Frequency (in %) of:			Number of rare alleles	Per cent homozygotes:	
		slow	medium	fast		observed	expected
			Sandwich Harbor/Mill Creek				
			Mill Creek Upstream				
4–8	143	36	28	36	1	51	34
9–13	98	17	34	49	0	47	38
14–19	138	24	33	43	2	55	35
20–25	133	25	33	42	4	44	35
26–31	96	14	41	45	4	58	39
32–45	209	13	38	49	4	57	40
46–70	214	16	38	46	3	57	38
			Mill Creek Subtidal				
5–19	153	16	31	53	4	47	40
20–33	87	22	28	50	0	56	38
34–81	146	15	30	55	3	55	42
			Station "C"				
20–25	30	25	34	41	1	—	—
26–31	53	22	32	46	2	47	36
32–56	63	35	27	38	1	45	34
			Station "B"				
26–31	30	30	32	37	1	—	—
32–44	66	26	34	40	1	60	34
			Station "A"				
12–25	74	24	33	43	1	59	35
26–39	22	49	32	19	1	—	—
			Sandwich Harbor Entrance				
9–13	49	24	36	40	1	52	35
14–19	69	18	42	40	4	55	37
20–25	47	53	22	25	1	65	39
26–31	75	54	19	27	1	43	40
32–53	69	47	27	26	3	30	36

TABLE I (*continued*)

Size range (in mm)	Number in sample	Frequency (in %) of:			Number of rare alleles	Per cent homozygotes:	
		slow	medium	fast		observed	expected
			Scorton Creek Upstream				
4–8	21	33	24	43	0	—	—
9–19	109	23	33	44	2	51	35
20–25	45	37	30	33	0	60	34
26–45	65	28	26	46	2	35	36
46–76	114	16	34	50	3	47	39
			Intermediate				
8–15	96	25	37	38	5	46	34
16–29	96	24	32	44	4	48	35
30–45	95	38	28	34	2	52	34
46–70	75	30	34	36	1	46	34
			Entrance				
12–20	89	29	32	39	1	50	34
21–30	20	30	37	33	0	—	—
31–40	35	47	23	30	0	46	36
			Two Jetty Sites Cape Cod Canal				
7–13	83	35	25	40	2	54	34
14–19	65	42	22	36	2	51	35
20–25	50	46	29	25	1	47	36
26–31	42	45	17	38	1	58	38
35–82	108	42	27	31	6	50	35
			Sandwich Harbor				
3–8	272	38	29	32	10	50	34
9–11	76	21	33	46	4	52	36
12–19	52	30	36	34	2	55	34
			Barnstable Harbor/Five Sites Mussel Point				
14–29	79	44	23	32	0	48	35
30–40	63	32	26	42	1	52	35

TABLE I (*continued*)

Size range (in mm)	Number in sample	Frequency (in %) of:			Number of rare alleles	Per cent homozygotes:	
		slow	medium	fast		observed	expected
Barnstable Harbor/Five Sites							
Mill Way Bridge							
18–25	31	39	22	39	1	—	—
26–32	19	40	34	26	0	—	—
33–45	50	53	24	23	1	45	39
46–72	50	56	22	22	1	43	41
Scudder's Lane							
20–25	33	33	27	40	0	—	—
26–31	15	47	20	33	0	—	—
32–85	58	45	23	32	3	54	36
Rendezvous Lane							
27–44	50	44	25	31	0	40	35
46–70	50	51	21	28	0	46	38
Barnstable Upstream							
19–39	30	33	18	48	0	—	—
40–78	28	52	21	27	0	—	—

RESULTS

The gene frequencies for the slow, medium and fast alleles for all localities are given in Table I. The number of the two rare alleles is also given; these alleles constitute only 1.1% of the total and show no pattern in their distribution over size classes or sites. Three mussels (in 4871) produced no discernible spots.

The pattern of allelic variation

From Table I we see that mussels in the different size classes from the Sandwich Harbor and Scorton Creek localities exhibit a wide variation in the frequency of S (from 13% to 54%) and a relatively constant ratio of M to F (about 2:3). The smaller mussels from all sites at these two localities generally have intermediate S frequencies, although there are differences between some of the classes at a site (note the contrast among the smallest three classes at the Mill Creek Upstream site). The larger mussels (those greater than 25 mm) show the greatest differences, and both Scorton Creek and Sandwich Harbor/Mill Creek have the *same* pattern of low frequencies of S at the upstream sites and high frequencies of S at

the entrance sites. This allelic variation is best summarized by the Chi-square tests of Table II, which compare the numbers of slow alleles among sites by combined size classes. For all mussels 25 mm and smaller, sites are essentially homogenous; the same test applied to the larger mussels reveals striking heterogeneity. Comparison of the larger mussels from the two entrance sites alone shows no significant difference; the two upstream sites are significantly different (S is 15% *versus* 21%), but converge to virtually identical frequencies when only those mussels over 45 mm are compared. The mussels from all the intermediate sites are concordant with this clinal pattern in that there are similar slow frequencies in the smaller mussels and the larger mussels have intermediate frequencies. Size classes between different sites may not be strictly comparable because the growth rates depend on such environmental factors as exposure time and food supply; however the class intervals were chosen *a priori* and not on the basis of tests of significance and therefore the tests and interpretations are conservative.

There is no good topographical counterpart to the Sandwich Harbor and Scorton Creek estuaries at Barnstable Harbor. Three of the sites—Scudder's Lane, Rendezvous Lane, and Mussel Point—were rock/sand beaches on the perimeter of the open water of the Harbor; in this they resembled the estuarine entrance sites, but differed in that they were far from the outlet of the Harbor to Cape Cod Bay. No mussels were found in the channels of the marsh except at the Barnstable Upstream site where they were very sparse; very few mussels were found alive and none was smaller than 19 mm. The Mill Way site was an isolated colony attached to a stone bridge about 100 meters from the Harbor.

The frequencies of the mussels from the Canal Jetty are essentially invariant throughout the size range. The Sandwich Jetty mussels were from a recent and temporary settlement, for no mussels were larger than 19 mm and most (87%) were less than 12 mm. Their growth rates were probably retarded since they were found high in the intertidal.

Although the ratio of medium to fast frequencies is much less variable among samples than is slow allele frequency, the ratio of medium to fast is not independent of slow frequency; the ratio is generally greater than 2:3 in those samples with a high slow frequency, and less than 2:3 where the slow frequency is low. This is true for Barnstable Harbor as well as Sandwich Harbor and Scorton Creek, and this observation may support the hypothesis that these alleles are not a neutral polymorphism.

Deviations from Hardy-Weinberg frequencies

Mussels in almost all size-classes from all localities show a significant excess of homozygote genotypes (SS, MM, and FF) over Hardy-Weinberg expectations. From Table I can be seen that for those samples of 35 or more individuals, only one (from the entrance to Sandwich Harbor) has fewer homozygotes than expected, and the average excess is 14%. There are three general explanations for this which do not involve selection: (1) presence of a null (or silent) allele, (2) persistent inbreeding, or (3) incorporation of the progeny of different populations into one sample.

TABLE II

Chi-square test for heterogeneity on allelic proportions for Sandwich Harbor/Mill Creek and Scorton Creek localities, by size; A = Mill Creek Upstream Intertidal; B = Sandwich Entrance; C = Scorton Creek Upstream; D = Scorton Creek Entrance (Jetty). Expected values in parentheses; $n_{ij} = n_{i.}n_{.j}/n_{total}$.

(1). Mussels Under 26 mm

	A	B	C	D	Sum
Slow:	267	96	97	54	514
	(262)	(95)	(102)	(56)	
Fast + Medium:	628	228	251	137	1244
	(633)	(229)	(246)	(135)	
Sum:	895	324	348	191	1758

Chi-square = 0.58; 3 d.f.; $P > 0.90$; 514/1758 = 29%

(2). Mussels Over 25 mm

	A	B	C	D	Sum
Slow:	150	144	74	42	410
	(239)	(66)	(83)	(22)	
Fast + Medium:	877	137	281	54	1349
	(788)	(215)	(272)	(74)	
Sum:	1027	281	355	96	1759

Chi-square = 190; 3 d.f.; $P < 0.001$

(3a). Two Upstream Sites (A and C); Mussels Over 25 mm

	Mill Creek	Scorton Creek	Sum
Slow:	150 (14.6%)	74 (20.8%)	224
	(166)	(58)	
Fast + Medium:	877	281	1158
	(271)	(297)	
Sum:	1027	355	1342

Chi-square = 7.56; 1 d.f.; $P < 0.01$

(3b). Two Upstream Sites (A and C); Mussels Over 45 mm

Slow:	68 (16.0%)	37 (16.6%)	105
	(69)	(36)	
Fast + Medium:	357	186	543
	(356)	(187)	
Sum:	425	223	648

Chi-square = 0.04; 1 d.f.; $P > 0.80$

TABLE II—(*Continued*)

(*4*). *Two Entrance Sites* (*B and D*);
Mussels Over 25 mm

	Sandwich Harbor	Scorton Creek	Sum
Slow:	144 (51.2%) (139)	42 (43.8%) (47)	186
Fast + Medium:	137 (142)	54 (49)	191
Sum:	281	96	377

Chi-square = 1.61; 1 d.f.; $P < 0.20$

A mussel heterozygous for a null allele will be scored as a homozygote, and if the null allele is lethal when homozygous, only test crosses will detect its presence. Under the hypothesis, the frequency of the null allele (r) can be estimated as

(1) $(1 - t)/(1 + t) = r$, where t is the ratio of observed heterozygotes to expected heterozygotes.

Inbreeding without change in gene frequency will produce an excess of homozygotes with frequency

(2) $\Sigma f(p_i) + (1 - f)p_i^2$, and the total number of heterozygotes will be reduced by a factor f (the inbreeding coefficient, Crow and Kimura, 1970). Adjustment by this parameter reduces the disparity between expected and observed by a factor of about 10.

Both the null allele and inbreeding models produce identical expected frequencies; in fact we obtain

(3) $f = 2r/(1 + r)$ or $r = f/(2 - f)$

and therefore can not be distinguished by the data of a single locus. For most of the samples, estimates of r range from 7% to 16% (or f from 0.13 to 0.28). These are high values.

The alternative explanation—that individuals in a sample originate from parental populations with disparate gene frequencies (the Wahlund effect)—seems more reasonable because (1) the extended pelagic existence favors dispersal and admixture, (2) striking differences in gene frequencies between adjacent size classes are observed, and (3) there are adjacent localities with characteristically high or low slow frequencies which are likely sources for the immigrants (Milkman and Beaty, 1970; Milkman, 1971).

In this model, we hypothesize that two parental populations produce progeny (with different Hardy-Weinberg proportions) which comprise a mixed population at the sampling site. The six genotypes in the mixed population may estimate five variables (the relative contribution of the parental populations and the frequencies of two alleles in each) and a minimum least-squares fit to the data obtained by

trial-and-error methods. However, once this is done, the system is still underdetermined and even if parental populations are assumed to have slow frequencies at or beyond the observed extreme values for slow, this model does not account for the data as well as the inbreeding model. Further, this mixed population model would also predict a decrease in homozygote excess in those size-classes with extreme values of the slow allele. Figure 3 does not show (with one exception) this trend. A further objection is that all other populations of *Mytilus*—including those putative parental populations with a nearly constant high or low slow frequency—show a consistent homozygote excess (Koehn and Mitton, 1972; Milkman and Beaty, 1970).

Environmental variation

Since our hypothesis is that the divergence in slow allele frequency is brought about by selection, we would like to correlate such environmental factors as exposure time, temperature, salinity, current and wave action, substrate, and associated biota with the pattern of genic variation. Environmental differences can be seen to affect the mussels even without electrophoretic assay, because their size range progressively increases as one goes upstream. Extensive search at both entrance sites did not produce any mussels larger than 53 mm at Sandwich Harbor and 40 mm at Scorton Creek. At the upstream sites there were many mussels per square meter larger than 60 mm.

The period of exposure does not seem important because the Mill Creek mussels were taken from both the high intertidal (exposure time in excess of five hours out of the twelve and one-half hour tidal cycle) and the subtidal zones, and the Scorton Creek upstream mussels were from the lowest intertidal (exposure time of about one hour). Mussels at both entrance sites were exposed for two to three hours.

At high tide mussels at all localities were covered by full-strength sea water (salinity of 32‰) and at low water the subtidal mussels were in effluent of minimum salinity of 10‰. The lowest values for sites A, B, and C were 12–14‰ and for the Sandwich Harbor entrance the minimum was 20‰. The Mill Creek Upstream mussels were never exposed to water of less than 26‰. Although the ultimate sources of the channels are fed by fresh water, the extensive marsh stores sea water like a sponge, and therefore the lower salinity values (less than 25‰) are recorded in the stream only after many of the mussels have been exposed. The temperature range of the water follows the same pattern in that the temperature was between 19° and 21° except in the few tide pools where it reached 30°, and in the channel at low tide where a maximum reading of 24° was once obtained on a hot sunny day. Air temperature over exposed mussels was close to ambient.

The chief difference between entrance and upstream sites is that mussels at the entrance were almost always attached singly to rocks buried in the sand, whereas upstream mussels were found in clumps or beds with direct attachment to one-another. At Mill Creek in particular, the beds were extensive and overlayed an ooze composed of fine silt, organic deposit, and broken mussel shells into which a collector would sink to his knees. At the Scorton Creek Upstream site the mussels were from mid-channel near low water, and had attached to large timbers which

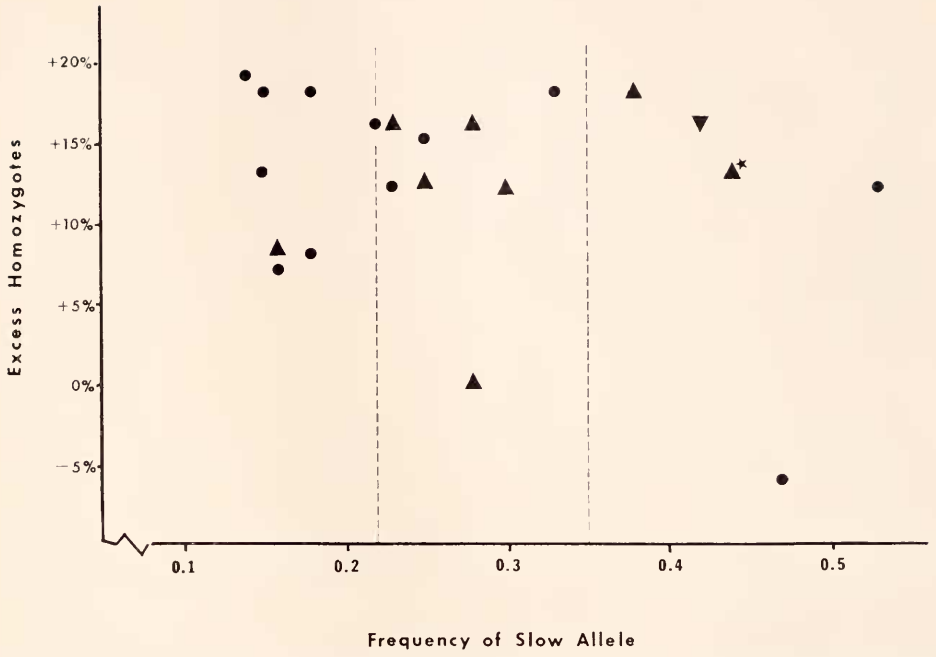

FIGURE 3. Deviations from Hardy-Weinberg expectations of homozygote frequencies *versus* slow frequencies in different size-class samples. Some adjacent size-classes (of Table I) with very similar genotype frequencies have been combined to give larger samples; $N > 65$ for all points except one ($N = 48$) from Scorton Creek (with star). Dotted lines delimit "intermediate" frequencies of slow; Mill Creek/Sandwich Harbor filled circles; Scorton Creek, filled triangles; Sandwich Canal Jetty, filled upside down triangle.

presumably protected them from shifting sand and provided additional anchorage. To summarize, at the Scorton Creek Upstream and Intermediate sites, and at the two Mill Creek sites, and to a lesser extent Stations B and C, mussels were in clumps (several handfuls would fill a bucket), and at the Entrance sites (and Station A) they were collected individually. Although there was discernible water movement either upstream or downstream through the channels throughout most of the tidal cycles, swift roiling currents were observed only in the three hour interval around low tide, where speeds attain five knots. Only the subtidal and low intertidal mussels were subject to the strong currents; presumably only the entrance sites would be exposed to wave action during a storm.

The associated biota seem to have little effect on the mussels themselves. No predators were seen except starfish and oyster drills at the Sandwich Canal Jetty; the small crabs found throughout the marsh were common in the mussel beds. In some samples platyhelminths and nematodes were inadvertently collected—most commonly in the upstream samples and rarely from the sand/rock beaches of the entrance sites. Only at the Barnstable Upstream site was *Modiolus* found with *Mytilus*.

Soft substrate, higher density, larger size, and "upstreamness" generally seem to go together, but no single factor stands out as the major determinant of gene frequency—particularly in view of the fact that an equivalent range of variation for any one factor could probably be found at either Sandwich Jetty (where the mussels were as dense and as large as upstream) or Barnstable.

The localities were studied only over the months of July and August, and it is likely that the greatest ecological contrasts may come in the winter months when productivity declines and freezing and ice impose a severe stress. A length of about 25 mm seems to mark the division of 1st and 2nd year animals (Harger, 1970; Milkman, 1971), and since allelic divergence begins at this size, a seasonal episode might be more important in selection than the differences which result from daily or tidal cycles.

Discussion

The divergence of slow allele frequency with increasing size strongly argues for selection; moreover it suggests a form of balancing selection which would maintain an enzyme polymorphism. Therefore these results parallel and substantiate the selectionist interpretation of Koehn and Mitton (1972) for the LAP locus in *Mytilus* and Koehn, Turano, and Mitton (1973) for the Tetrazolium oxidase (*To*) locus in *Modiolus*. There are however some important differences. First, the *Mytilus* populations described by Koehn and Mitton have a nearly constant S frequency (11%–14%) and the major component of the differences between the sites is the degree of homozygote excess (the statistical tests were made on the genotypes of FF, FX, and XX, where X = M or S). There is no evidence for a clinal pattern at the four sites; whether there is genetic heterogeneity between size classes is not reported.

Modiolus populations at both a high intertidal and low intertidal site have size-dependent changes in Tetrazolium oxidase frequencies (Koehn *et al.*, 1973). These data are pertinent because these sites may be ecological counterparts of the upstream and entrance sites of *Mytilus*, and Koehn and Mitton (1972) have shown that *Modiolus* has a pattern of genic variation similar to *Mytilus* in the Nissequoque River. The TO allele frequencies are essentially constant over size classes and between sites, but at both sites there is an increase with age in the heterozygote relative to the two homozygotes. In *Mytilus* we find—in general—variation in allele frequencies and no trend in Hardy-Weinberg deviations; but for both species, *there is an initial homozygote excess.*

For *Modiolus*, Koehn, Turano, and Mitton (1973) suggest an alternative explanation for this excess, namely selection against heterozygotes at the larval stage prior to settlement. This seems unlikely in the case of the LAP locus in *Mytilus* because there are three heterozygote genotypes which would appear to be equally disadvantaged.

The cause of the persistent Hardy-Weinberg deviations is important in that our conclusions as to *what* is being selected depend upon this issue. If the mussels at each site are immigrants from two (or more) parental populations, then all genes are linked (Milkman and Beaty, 1970) and the LAP locus is a marker of parental origins. Selection is acting in this case on an entire genome, and it is for this

reason we anticipate an increasingly better fit to Hardy-Weinberg proportions with age.

If mating is non-random but essentially panmictic, then selection could be acting on the LAP locus and closely linked loci. If the populations are panmictic and all genes are in linkage equilibrium (and the deviations are due to a silent allele), then a strong case could be made for selection at the LAP locus itself.

None of these alternatives is strongly supported by the evidence. The three mussels which produced no stain reaction support the null-allele theory, but they could have resulted from a physiological inactivation rather than a null genotype, and there is the further onus of justifying how this allele could persist in high frequency (around 12%). Inbreeding (or assortative mating) is very plausible for many animal populations, but the gametes of *Mytilus* are spawned into open water. Microgeographic variation, or more likely, temporal variation in spawning correlated with genotype could be responsible for non-random mating. The difficulties with the mixed-population model have already been discussed; of course, to reject it as the explanation for homozygote excess is not to insist that all individuals at a site originate from one population.

As in most studies of this kind, the mussels were collected over a period of time which was brief compared to the life-span of the animal, and the assumption has been made that the pattern we observe is persistent and not due to differential colonization in prior years. R. Milkman has studied a *Mytilus* population in the Cape Cod Canal for three successive years, and reports a consistent colonization pattern over this period. Further, differential colonization as a result of habitat selection would also imply an adaptive role in maintaining the polymorphism.

Although the problem of excess homozygotes has not been resolved, the *Mytilus* data taken as a whole strongly implicate selection as the agent responsible for the pattern of genic variation. The clear divergence of LAP allele frequences with increasing size at two separate, but ecologically similar, localities is good evidence for an adaptive polymorphism in a natural population.

I would like to thank Dr. Roger Milkman for suggesting this study and for his help on numerous occasions. The technical assistance of R. Zeitler and the use of some of the facilities of the Marine Ecology course at Marine Biological Laboratory are gratefully acknowledged. The work was supported in part by the Biological Sciences Development Grant, GM 2591, from the NSF to the University of Iowa and NIH GM 18967 to R. Milkman.

Summary

1. Samples of the mussel, *Mytilus edulis,* were taken from 16 sites on the northern shore of Cape Cod. The mussels were measured and their genotypes at the leucine-amino-peptidase (LAP) locus determined by electrophoresis.

2. At two separate estuarine localities, Sandwich Harbor and Scorton Creek, a pronounced cline in slow allele frequencies in the larger mussels was found,

with upstream sites showing characteristically low frequencies (about 15%) and downstream (entrance) sites having high frequencies (45% to 55%). Mussels smaller than 26 mm had intermediate (22% to 35%) slow frequencies.

3. This clinal divergence with increasing size strongly argues for an adaptive polymorphism. Mechanisms for the observed excess of homozygote genotypes in almost all samples are also discussed.

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